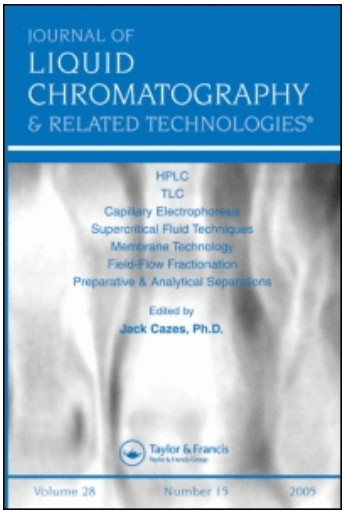

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APPLICATION OF SEPHADEX LH-20 CHROMATOGRAPHY FOR THE SEPARATION OF CYANOGENIC GLYCOSIDES AND HYDROPHYLIC PHENOLIC FRACTION FROM FLAXSEED

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ABSTRACT

A column chromatographic method, using Sephadex LH-20, for separation of cyanogenic glycosides and hydrophylic phenolic compounds from flaxseed is described. Results so obtained indicate that this method can be used as a first step in chromatographic separation and purification of linustatin, neolinustatin and phenolics compounds from flaxseed.

INTRODUCTION

Flaxseed (*Linum usitatissimum L.*) contains a number of anti-nutritional factors, amongst which cyanogenic glycosides are of most concern (1). Linustatin and neolinustatin have been identified as the major cyanogenic compounds in

flaxseed (2). Their content in flaxseed may be as high as 350 and 200 mg/100 g, respectively (3). Phenolic acids are the best-known group of phenolic compounds present in flaxseed (4,5). However, little is known about other classes of phenolic compounds present in the oilseed. As a first step in identification of such compounds, it was necessary to device a simple procedure for their isolation.

Application of Sephadex LH-20 gel filtration for separation of cyanogenic glycosides and hydrophylic phenolic compounds from flaxseed was intended. This method has been used previously for separation of phenolic compounds and glycosides from other plant materials (6-8).

MATERIALS AND METHODS

Defatted flaxseed was extracted with 80% (v/v) ethanol over a 1 h period at a seed to solvent ratio of 1:5 (w/v) at 65-70°C (9). The hydrophilic fraction of the extract was obtained by butanol/water (1:1, v/v) separation (6). The bottom aqueous layer in the separatory funnel was recovered and then lyophilized. Two grams of the residue so obtained was dissolved in methanol and applied to a C16/100 column (Pharmacia LKB, Uppsala, Sweden) packed with Sephadex LH-20 and eluated with methanol. Fractions (6 mL) were collected using a LKB Bromma 2112 redirac fraction collector (Pharmacia LKB, Uppsala, Sweden). The absorbance of methanolic solutions from each tube was measured at 280 nm. In addition, absorbance values at 490 nm and 726 nm were read after colour development reactions for sugars (10) and phenols (11), respectively. Eluates were then pooled into 5 fractions and weighed. UV spectra were obtained using

a Hewlett Packard 8452A diode array spectrophotometer. Fractions were also examined on TLC silica gel plates (Sigma, St. Louis, MD) using a chloroform/methanol/water (65:35:10, v/v/v) system for glycosides (12) and a butanol/acetic acid/water (3:1:1, v/v/v) system (13) for separation of phenolic compounds.

Sugar compounds were visualised after spraying the developed TLC plates with 0.1% orcinol in 75% sulphuric acid and heating for 5 min at 120°C (14). For phenolic compounds, plates were sprayed with a ferric chloride-potassium ferricyanide solution (15). In the case of sugars, raffinose and sucrose (Sigma, St. Louis, MO), as well as cyanogenic glycosides, linustatin and neolinustatin standards were also applied to TLC plates. The two cyanogenic glycosides were prepared as described elsewhere (16). For phenolic compounds, synapic acid, ferulic acid, nordihydroguaiaretic acid (NDGA), and glucopyranosyl sinapate were used as standards. Sinapic and ferulic acids are the main phenolic acids of flaxseed (4,5), and chemical structure of NDGA is very similar to that of flaxseed lignan (17). The standard glucopyranosyl sinapate was isolated from canola meal (18).

RESULTS AND DISCUSSION

The hydrophilic fraction of flaxseed extract was subjected to Sephadex LH-20 chromatography. The chromatogram (Figure 1) exhibited a large and two small peaks at 280 nm, the typical absorption wavelength for aromatic compounds, three maxima at 726 nm for phenolics, after color development (one large and two

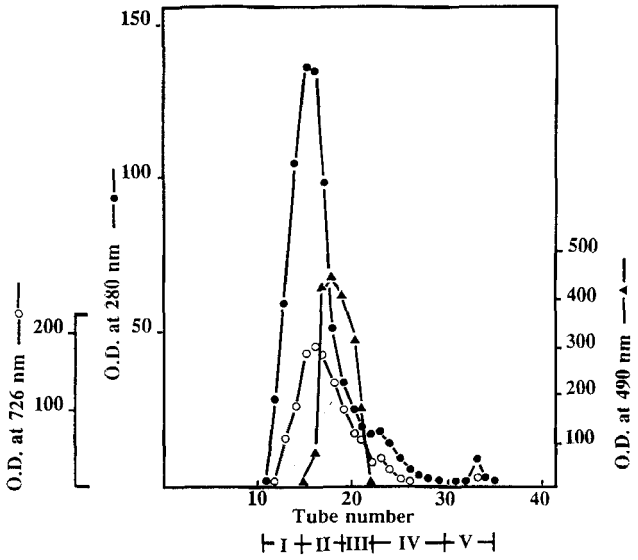

Figure 1. Sephadex LH-20 chromatography of hyhrophilic compounds from flaxseed separated on a 16/100 Pharmaica column using methanol as the eluting solvent.

small), and one maximum at 490 nm for sugars. The maxima for phenolics coincided with the UV peaks. The maximum observed for sugars occurred after the first phenolic peak.

Accordingly, five fractions (I-V) were separated after Sephadex LH-20 chromatography. These fractions contributed: I, 15.6%; II, 63.6%; III, 16.7%; IV, 3.6%; and V, 0.5% to the total amount of hydrophilic extracts.

Spraying of the TLC plates with the orcinol reagent indicated the presence of linustatin and neolinustatin in fractions II and III (Figure 2). In addition, raffinose was observed in fractions II, III, and IV and sucrose was present in

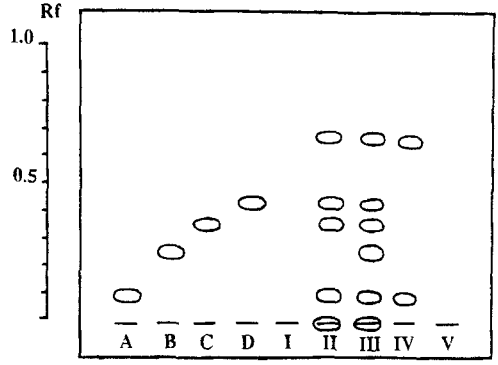

Figure 2. TLC separation of flaxseed sugars and glycosides. A silica gel phate and a solvent system consisting of chloroform/methanol/water (65:35:10, v/v/v) were used. Standards were: A, raffinose; B, sucrose; C, linustatin; D, neolinustatin. Fractions I to V were separated on a Sephadex LH-20 column.

fraction III. The most polar sugars (spot on the base line) were present in fractions II and III. Presence of less polar sugar (Rf=0.67) in flaxseed has already been reported in the literature (15).

TLC analysis showed that the isolated fractions contained numerous phenolic compounds (Figure 3). They were present in all five fractions but were dominated in fractions I to III. Polarity of the phenolic compounds was very different. Two polar compounds were present infraction I, one giving a long spot on the TLC plate. Fractions II, III, and V contained only simple phenolics. Three phenolics were visualized in fraction IV. Rf values of all hydrophylic phenolics were less than Rf of sinapic acid, ferulic acid and NDGA. However, Rf of one compound in fractions II-IV was very close to the Rf value of the glucopyranosyl sinapate. The hydrophylic phenolic fraction from flaxseed does

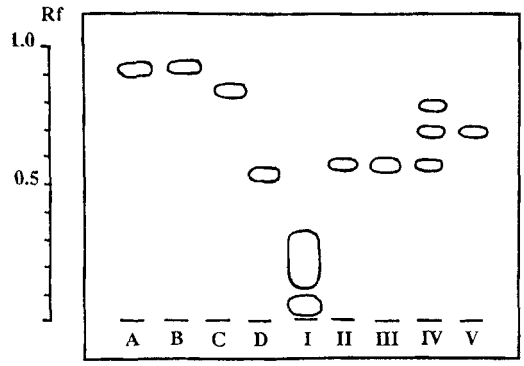

Figure 3. TLC separation of flaxseed phenolics. A silica gel plate and a solvent system consisting of n-butanol/acetic acid/water (3:1:1, v/v/v) were used. Standards were: A, sinapic acid; B, ferulic acid; C, nondihydroguaiaretic acid (NDGA); D, glucopyranosyl sinapate. Fractions I to V were separated on a Sephadex LH-20 column.

not show the presence of any free phenolic acids. Most probably glycosides of phenolics occur in this fraction.

UV spectra (Figure 4 and Table 1) of fractions I to III indicated similar absorption bands with maxima at 286, 284, and 282 nm and a shoulder at 308 nm. However, fraction III showed an additional shoulder at 290 nm. Absorption maxima for UV spectra of fractions IV and V were noted at shorter wavelengths of 270 and 274 nm. Fraction IV exhibited shoulders at 290 and 312 nm, fraction V at 282, 334 and 376 nm.

The chromatographic method described in this work may be employed as a preliminary step in separation of cyanogenic glycosides from flaxseed. Polar sugars obtained in fractions II and III can be easily removed by dissolving the

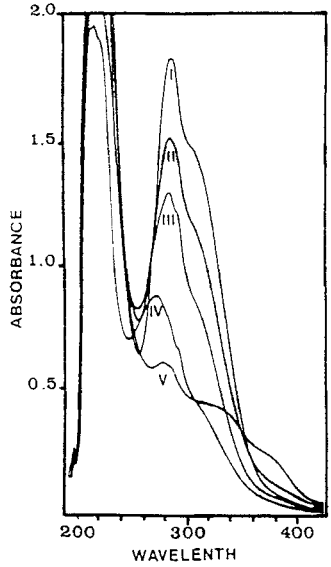

Figure 4. UV spectra of flaxseed extract fractions I to V separated on a Sephadex LH-20 column.

TABLE 1
UV spectral data of different fractions of flaxseed extract (λ_{max}, λ_{sh}, nm).

Fractions	λ_{max}	λ_{sh}
I	286	308
II	284	308
III	282	290, 308
IV	270	290, 312
V	274	282, 334, 376

extract in a chloroform/methanol mixture (15) and linustatin and neolinustatin may be further separated and recovered in pure forms using a reverse phase (RP) (2) or silica gel column chromatography (16). This would also allow the separation of compounds, especially individual pure compounds from fractions II and III and polar compounds from fraction I.

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